Grasses and Legumes for Cellulosic Bioenergy 12

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"[G]rassland agriculture, like agriculture by any other name, is subject to modification by the sweeping influence of social and economic change. It cannot deny or ignore the effects of varying systems of land tenure, . . . the impact of shifts in national policies, or the political trends resulting from these and other factors that affect the lives of people on the land. . . .

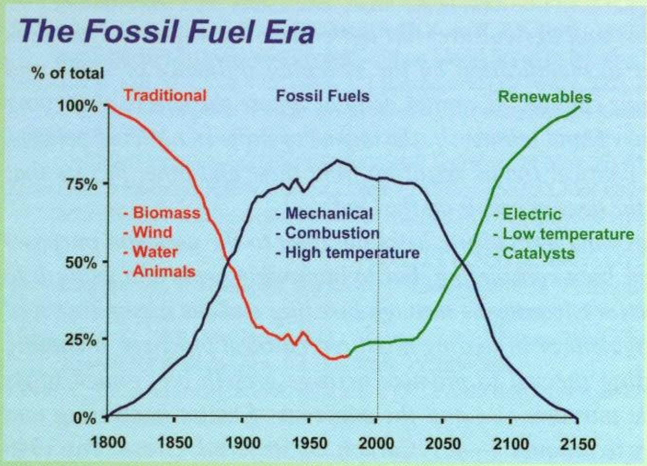
"[G]rassland agriculture looks not only to the uses and purposes we have been considering, but to improving grass and using it to still better advantage—through breeding and the use of improved species, alone or in seeding mixtures; cultural practices, including amending the soil to promote herbage growth best suited to its specific purpose; . . . and the adoption of better harvesting and storage techniques."—P.V. Cardon, in Stefferud, Grass: The 1948 Yearbook of Agriculture, *pp. 2, 5*

Human life has depended on renewable sources of bioenergy for many thousands of years, since the time humans first learned to control fire and utilize wood as the earliest source of bioenergy. The exploitation of forage crops constituted the next major technological breakthrough in renewable bioenergy, when our ancestors began to domesticate livestock about 6000 years ago. Horses, cattle, oxen, water buffalo, and camels have long been used as sources of mechanical and chemical energy. They perform tillage for crop production, provide leverage to collect and transport construction materials, supply transportation for trade and migratory routes, and create manure that is used to cook meals and heat homes. Forage crops—many of which form the basis of *Grass: The 1948 Yearbook of Agriculture* (Stefferud, 1948), as well as the other chapters of this volume—have composed the principal or only diet of these draft animals since the dawn of agriculture.

HISTORY OF CELLULOSIC ENERGY

Throughout most of human history, wood, peat, and forage crops have served as ideal renewable bioenergy feedstocks. Their biomass is derived from photosynthesis, energy obtained directly from the sun, representing the most abundant source of renewable energy on the planet. Until the development of fertilizer applications in the early 18th century, fertilization of bioenergy crops was accomplished by nutrient mineralization in soil or by addition of human or livestock waste materials. Fossil fuels did not contribute to forage

The human race is at an important crossroads, one that will determine our ability to sustain societal structures and lifestyles at the level and function that we have enjoyed for many years. As we wean ourselves of dependency on fossil fuels, we are gradually rediscovering the fuels of our ancestors. The promise and optimism for a biofuel-based economy and lifestyle resides in combining these ancient fuel sources with modern technologies that make them more efficient, renewable, and sustainable. Scientists, politicians, and industry leaders have a great responsibility to lead societies in the correct choices to improve and develop a wide array of alternative renewable fuels to serve the needs of geographically and culturally diverse societies.



Trends in human use of traditional fuels, fossil fuels, and renewable fuels from 1800 to present and predicted to the mid–22nd century. (Source: Ewald Breunesse, Shell Netherlands, 14th IAMA World Food and Agribusiness Forum, Montreaux, Switzerland. 12–15 June 2004.)

production or utilization until steamships and steam-powered locomotives could transport livestock and hay across oceans and continents. Until fossil fuels entered the picture, forage crops were an essential component of a sustainable lifestyle and society, defining the limits of population growth, distribution, density, and human consumption rates.

Throughout human history, energy density has always been the principal driver of choices among various sources of energy. Thus, wood, where available, was favored by early humans over peat and manure for heating and cooking. With the development of mining and transportation technology, the more energy dense coal replaced wood as the principal source of heat in the 17th century. Likewise, petroleum products fueled new technological advancements, placing petroleum at the forefront of the energy industry beginning in the late 19th century. If affordable and socially acceptable solutions could be found for the two principal challenges associated with nuclear fission—risk of radiation contamination and effective waste disposal—nuclear energy might render moot all current discussions of bioenergy.

The transition from fossil fuels to renewable and bio-based energy sources may be slow, expensive, and perhaps traumatic for many countries throughout the world. Fortunately, renewable and bio-based fuels have been constant sources of energy throughout modern human history, sometimes benefiting from technological advancements. Furthermore, research on renewable and bio-based energy products has expanded significantly in the last 20 years, creating new options for energy generation across the globe. This chapter explores many of the current and theoretical future technologies that will facilitate our transition from a fossil fuel–based society to one that can produce food, clothing, fuel, and other products in a manner that matches sustainable production, planetary limitations, human population growth, and human lifestyles and living standards.

CELLULOSIC CONVERSION TECHNOLOGIES

THERMOCHEMICAL PROCESSES

The simplest process for obtaining energy from biomass is direct combustion, in which the full energy content of the biomass is released as heat during the conversion of organic material plus oxygen to carbon dioxide plus water. Typically, biomass has a relatively low energy density and contains substantial amounts of ash and water, making it a less-potent fuel than coal and other hydrocarbons. In current energy production systems, combustion of biomass is usually performed by co-firing relatively small amounts of biomass with much larger amounts of coal. At typical co-firing rates of 5% biomass, improved combustion properties of both fuels are obtained, along with a reduction in NO_x and SO_x emissions. Heat of combustion is usually captured via steam turbines and then converted to electricity.

Several alternative thermochemical processes can produce liquid fuels from biomass; liquid fuels for transportation form the most critical energy need in the United States and most developed countries. One such process is *gasification*, a complex partial combustion of organic material conducted at high temperature (>1300°F [700°C]) under limiting amounts of oxygen. Products include synthesis gas (*syngas*, a mixture of hydrogen and carbon monoxide), along with carbon dioxide and water. Syngas can be burned in a gas turbine to produce electricity or used as a feedstock for synthesis of liquid fuels and industrial chemicals (e.g., methanol). A more extreme thermochemical process is *pyrolysis*, in which the biomass is converted at high temperature and in the absence of oxygen (Bridgwater, 2004). Biomass pyrolysis can produce alkane gases, carbon monoxide, carbon dioxide, and hydrogen, along with a solid char. Modifying reaction conditions can shift reaction products away from these gases and toward pyrolysis oils, which may be refined to liquid fuels, particularly a synthetic diesel fuel (Huber et al., 2006). The pyrolysis reactions themselves are slightly endothermic, so heat must be provided to sustain the pyrolytic reactions. Moreover, the biomass particles must be ground to a fine size to enhance heat transfer required for rapid reactions. Engineering, managing, and optimizing complex pyrolysis reactors are complicated by this heat transfer requirement and the need to remove the char waste product.

BIOFUEL PRODUCTION VIA FERMENTATION

While fermentation of starch-based products, such as grain crops, for biofuel production has become common, commercial-scale production of liquid fuels from the fermentation of cellulosic biomass continues to face significant scientific and economic challenges. Fermentation of cellulosic biomass requires the breakdown of complex cell-wall components into its component monosaccharide sugars (individual sugar molecules that are the building blocks of cellulose and hemicellulose) before yeast and other microorganisms can ferment the biomass to produce liquid biofuels (Lynd et al., 2002). These free sugars are converted to ethanol and other biofuel products by microbes under oxygen-free conditions. While successful conversion of cellulosic biomass to biofuels has been demonstrated, the economics remain marginal because breaking down cellulosic biomass into free sugars and obtaining high yields of biofuels from the diversity of monosaccharides (simple sugar molecules) found in cell-wall polysaccharides (complex sugar molecules) remains difficult.

Conversion of cellulosic biomass to fermentable sugars requires two steps: (i) pretreating cellulose and hemicellulose to increase enzyme accessibility, and (ii) enzymatic breakdown of the polysaccharides to free sugars (*saccharification*). Lignin is the most important inhibitor that must be removed; however, hemicellulose can also be removed through pretreatment to increase the access of enzymes to cellulose. Of the various pretreatments that have been explored, dilute acid–high temperature pretreatment and ammonia fiber explosion (AFEX) are the most highly developed (Mosier et al., 2005). Acid pretreatment increases the accessibility of the biomass to enzymatic breakdown of cellulose by cleaving most of the hemicellulose into free sugars and degrading much of the lignin. This pretreatment is very effective for increasing cellulose breakdown potential, but it requires substantial investment in reactor equipment (acid-resistant stainless steel for pipes and pumps) and energy for generating high temperatures (350–390°F [180–200°C]). Also, if reaction conditions are not carefully controlled, pentose sugars (xylose and arabinose) in hemicellulose can be converted into toxic products that poison the microbes subsequently used for fermentation. The AFEX method involves exposing biomass to pressurized ammonia, followed by rapid pressure release. This results in a physical rupture and expansion of the cell-wall matrix structure, thereby increasing accessibility for cellulose-degrading enzymes. Because ammonia is a weak base, it also helps swell the cellulose fibers and break ester cross-links, the chemical bonds between lignin and hemicellulose in grasses. Additional hurdles to commercial-scale production include the cost of building pressure vessels and the difficulty in designing more efficient continuous-flow AFEX systems, rather than relying on batch processing systems.

The enzymatic saccharification step to convert pretreated cellulose to free glucose requires a minimum of three different enzymes. While significant progress has been achieved in improving the activity of these enzymes, enzyme costs remain a major constraint for cellulosic biofuels. Breakdown of hemicelluloses for biofuel production is currently not feasible on a commercial scale, largely because the full spectrum of enzymes needed is not yet available. For acid-pretreated biomass, these enzymes are not required because hemicellulose can be broken down by acid pretreatment; however, the AFEX procedure requires appropriate enzymes for hemicellulose hydrolysis. Such enzymes would avoid the production of fermentation inhibitors during acid pretreatment and perhaps reduce the cost of pretreatment.

Once cellulosic biomass has been broken down into its component sugars, microorganisms are needed to ferment the diversity of sugars to the desired biofuel. Yeasts currently used for ethanol production from corn grain can only ferment glucose (from starch) and other hexoses (6-carbon sugars); therefore, these strains can utilize glucose released from cellulose but cannot ferment the hemicellulosic pentose sugars xylose and arabinose (5-carbon sugars). Genetic engineering has been used to modify yeast strains to effectively use xylose, with current efforts devoted toward use of the other nonglucose cell-wall sugar components, albeit at slower rates. Other naturally occurring and genetically modified organisms are being evaluated for fermentation of cellulosic biomass. Rate of fermentation, tolerance of biofuel product accumulation, nutrient requirements for growth, and other challenges currently limit commercial-scale use of such microbes. Because efficiency of enzymatic breakdown of cellulose is inhibited by the accumulation of glucose, simultaneous enzymatic saccharification and microbial fermentation (SSF) in the same vessel is necessary for most conversion systems.

A further advance anticipated to improve conversion efficiency is consolidated bioprocessing (CBP). In such a system, the microorganism (certain anaerobic bacteria) not only ferments cell wall sugars to biofuels but also produces the enzymes needed to break down cellulosic biomass into free sugars, thereby eliminating the need for a separate enzyme production process. Consolidated bioprocessing has several theoretical advantages (single reactor configuration, potential for hemicellulose utilization) over conventional SSF systems, but it faces hurdles of its own. In particular, these bacteria produce substantial amounts of other fermentation products (acetic acid, lactic acid, and hydrogen gas) that reduce ethanol yield. Moreover, these bacteria have poor tolerance of ethanol, so much so that reducing the ethanol concentration to acceptable levels has proven to be economically unacceptable. Reducing formation of fermentation products improves ethanol tolerance, suggesting these bacteria are more sensitive to organic acids. If this is the case, improving ethanol yield by strain selection or genetic manipulation should also improve ethanol concentrations in the reactor, perhaps to economically favorable levels.

HERBACEOUS CELLULOSIC BIOMASS CROPS

Around the world, many plant species have historically contributed, and will continue to contribute, to human bioenergy needs. Economic analyses routinely point to sustainably high biomass yield (including persistence and pest resistances) as the most important trait(s) for the development of bioenergy crops to effectively replace significant amounts of fossil fuels (Perlack et al., 2005). This means that bioenergy crops must be carefully matched to local environments, accounting for adaptation, pest resistance, stress tolerance, and photosynthetic efficiency issues. Layered on top of this are issues of invasiveness and protection of increasingly scarce and threat-

WHAT IS CELLULOSIC BIOMASS AND WHY IS IT SO RESISTANT TO DEGRADATION?

Cellulosic biomass refers to all aboveground plant biomass except seeds harvested for grain. The largest single biochemical constituent of biomass is the polysaccharide cellulose; hence, the term *cellulosic* biomass. All plant cell walls contain a mixture of three polysaccharides: cellulose, hemicellulose, and pectin. Cellulose is the simplest of the three in composition, consisting of only glucose units, whereas hemicellulose and pectin are actually groups of polysaccharides containing a large variety of sugars and linkages. Hemicellulose is generally the second most abundant cell wall polysaccharide, about half the concentration of cellulose. Pectin is a minor component of grass cell walls, but in legumes and other dicots, pectin concentration can equal hemicellulose levels. The cell wall provides structural rigidity to plant tissues, in addition to serving other important roles such as a defense against pathogen entry. Plant cell walls differ in thickness and composition among taxonomic groups, change occurs with physiological maturity, and walls vary among the cell types that comprise plant tissues. Most plant tissues that develop thickened walls also incorporate lignin, a phenolic polymer, into the cell wall. This is illustrated by the red staining (indicator of lignin) of all tissues in the maize stem rind except for phloem (phl) and some parenchyma immediately under the epidermis, shown in Fig. 12–1a. While this pattern of almost universal thickening and lignification of stem tissue walls is generally true for all grasses, in legumes only the vascular tissues and very mature pith parenchyma cells lignify, leaving many tissues lignin free.

Cell walls can be characterized as cellulose fibers encased in a matrix of hemicellulose and lignin. The hemicellulose must be removed, either chemically or enzymatically, to allow access to cellulose fibers by cellulose degrading enzymes. More important, lignin blocks enzyme access to both cellulose and hemicellulose. The dramatic negative impact of lignin on cell wall polysaccharide degradation is illustrated by the complete degradation of nonlignified phloem tissue in maize rind, but only partial thinning of lignified tissues (Fig. 12–1b). Therefore, removal of lignin is a key step in the production of cellulosic ethanol.

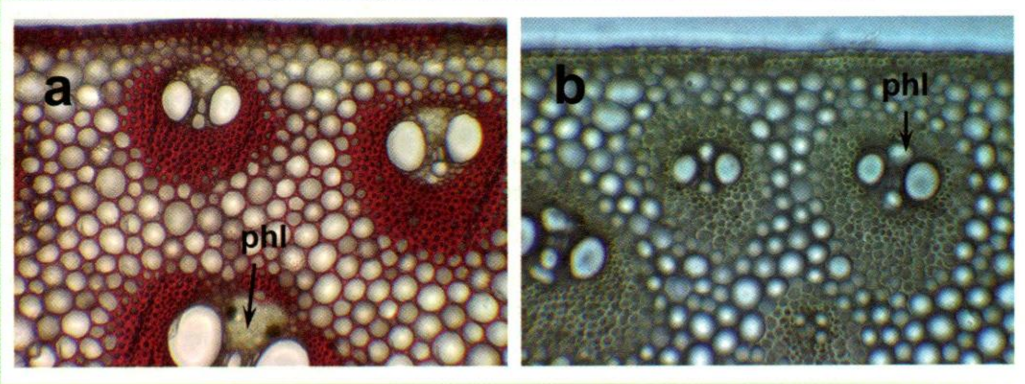


Fig. 12–1. Rind tissues of a corn stem harvested at physiological maturity that have been stained to highlight (a) the presence of lignin in red and (b) the limited cell wall thinning (degradation) that occurs during in vitro rumen digestion process. (Photos provided by H. G. Jung)

ened natural areas. Indeed, the global economy, powered by fossil fuels, has allowed humans to create artificial plant migrations that could never have occurred without human intervention. While the popular press and scientific literature are rife with negative examples of environmental degradation, habitat loss, and agricultural pests resulting from introduction of invasive species, there are some positive stories. Introduction of the European perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) and the previously unproductive and economically useless Monterey pine (*Pinus radiata* D. Don.), for example, transformed the dairy and forestry industries of New Zealand to be of global economic significance. There is a need for increased collaboration, communication, and compromise between the agricultural and environmental scientific communities to develop bioenergy crops that optimize human needs, including food, clothing, fuel, conservation, and recreation.

Perennial Grasses and Legumes

Warm-Season Grasses

Sugarcane (*Saccharum* spp.), including specific varieties referred to as "energy cane," is the most notable success story in the development of a proven perennial cellulosic bioenergy crop. Sugarcane and energy cane are the most important sources of biomass for ethanol production in Brazil, with production of 460 million tons (417 metric tons) of cane, 31 million tons (28 metric tons) of sugar, and 4.3 million gallons (16 million L) of ethanol in 2005. The fibrous residue, *bagasse*, is used by most sugar mills to produce heat, steam, and electricity to power the sugar milling process. While traditional sugarcane varieties have been used to develop this industry in Brazil, new varieties of energy cane are becoming more widely available and useful. Energy cane varieties have reduced moisture and sugar, but increased fiber, at harvest compared with traditional sugarcane. Increased biomass yield, deriving from a combination of genetics and management, is a principal advantage of energy cane varieties. One distinct disadvantage is the reduced flexibility in use of energy cane biomass, because of its reduced sugar content.

Switchgrass (*Panicum virgatum* L.) has received more attention as a potential bioenergy crop than any other perennial plant in the United States, thanks to its status as the herbaceous model species for the U.S. Department of Energy's Feedstock Research Program, initiated in 1992 (Sanderson et al., 2007). Switchgrass is a native of the historical tallgrass prairie and oak savanna ecosystems that once stretched from southern Texas to the Dakotas, including much of the modern Corn Belt of the United States. Less than 1% of these ecosystems exist today, but these prairie and savanna remnants have served as in situ gene banks, preserving a vast amount of genetic diversity within switchgrass and many other plant species. Today, switchgrass can be found in a wide range of habitats east of the Rocky Mountains and generally south of 55°N latitude.

Breeding, genetics, and agronomic research on switchgrass began during the 1930s in Lincoln, Nebraska, collaboratively between the USDA Agricultural Research Service (ARS) and the University of Nebraska. Research on switchgrass for pasture or hay production, soil conservation and prairie restoration, or basic biology remained at a relatively low level until U.S. Department of Energy funding created a large number of switchgrass research centers across the eastern two-thirds of the United States (Sanderson et al., 2007).

Numerous cultivars of switchgrass fall into two categories: (i) *natural-track cultivars*, which are seed increases from random plants originating from a source-identified prairie remnant, or (ii) *improved cultivars*, which originate from a breeding program. As of this writing, all improved or bred cultivars originate from breeding programs in Nebraska or South Dakota, generally originating from seeds and plants collected in the Great Plains region of the United States. Improved cultivars represent selections of a small number of defined traits, including biomass yield, digestibility, seed size, and disease resistance and are generally no more than two or three sexual generations removed from wild switchgrass collections. Natural-track cultivars represent a much broader geographic region, including many source-identified sites ranging from the Great Plains to the Atlantic Seaboard and from the Dakotas to Texas.

Switchgrass has two main ecotypes and a wide range of lesser ecotypes within the main ecotypes, upland and lowland. As the names suggest, the lowland ecotype was originally found on

Lowland (left) and upland (right) types of switchgrass are distinctly different from each other in overall appearance. Note the bluish coloring, thicker stems, more upright leaves, and later heading of the lowland plant on the left. (Photo by M.D. Casler)

flood plains and riparian zones subject to occasional flooding and/or waterlogging. The upland ecotype was originally found in upland areas that were not subject to flooding and were often prone to drought. Plants of the upland and lowland ecotypes are morphologically and genetically distinct from each other. Generally, lowland plants have a later heading date (date at which the head emerges from the boot), taller plant height, larger and thicker stems, few stems per plant, more upright leaf blades, and a more bluish cast than upland plants. Upland and lowland plants can be easily crossed with each other, and intermediate types exist in nature, suggesting upland × lowland crosses have occurred in natural ecosystems, despite the large difference in heading date between upland and lowland ecotypes.

Identification of switchgrass as a model species for bioenergy production in 1992 resulted in a critical mass of scientific research that has significantly reduced the expected cost of biomass production from switchgrass (McLaughlin and Kzsos, 2005). Reductions in input costs derive from (i) selection of adapted cultivars with the best long-term production potential, (ii) improved stand establishment and optimization of harvest timing and frequency, and (iii) reduction in nitrogen fertilizer applications. Switchgrass cultivars vary widely in a number of important adaptation traits, including heat and cold tolerance and response to photoperiod, and should not be exported more than one hardiness zone north or south of their origin. Establishment of switchgrass has been improved by research identifying appropriate planting dates, seeding rates, seed scarification or stratification techniques, selection for reduced seed dormancy, herbicides, and interplanting switchgrass with row/nurse crops. With adequate moisture, switchgrass will produce approximately 1 ton of dry matter per acre for each 20 lbs of nitrogen applied ($\sim$1 Mg/ha for each 10 kg N/ha).

Big bluestem (*Andropogon gerardii* Vitman), indiangrass [*Sorghastrum nutans* (L.) Nash], and prairie cordgrass (*Spartina pectinata* Bosc ex Link) are other dominant members of the tallgrass prairie and closely related ecosystems. These three grasses also have high biomass yield potential and are candidates for bioenergy crop development. A relatively small number of cultivars exist for each of these species, reflecting the relatively small amount of effort directed toward research on germplasm resources, breeding and genetics, and agronomic production. Big bluestem produces significantly more biomass per unit of nitrogen than switchgrass, and its feedstock quality may be higher than switchgrass, suggesting a need to expand research on this species.

Just as switchgrass was chosen as a model energy crop in the United States, a warm-season C_4 grass from the *Miscanthus* genus was selected for study in the European Union (EU) (Jones and Walsh, 2001). Native to East Asia, where they are used for thatching and paper production, *Miscanthus* species were originally brought to Europe as ornamentals. One such specimen, *Miscanthus* × *giganteus* (Greef et. Deu), was brought to the Netherlands in the 1930s because of its remarkable height (10–14 feet [3–4 m]) and fountainlike foliage. Some *Miscanthus* species can cross with sugarcane, thereby enhancing cold tolerance and disease resistance in the latter. *M.* × *giganteus* looks and behaves like a cool-season sugarcane, but without the high sucrose content. Rediscovered in the search for an "ideal" bioenergy species, this sterile hybrid of two other *Miscanthus* species (*M. sinensis* and *M. sacchariflorus*) was cloned and evaluated throughout the EU during the 1990s and is now used for heat and electricity production.

Like switchgrass, *Miscanthus* is broadly adapted, growing in USDA hardiness zones 4 to 10, roughly Canada to Mexico, and preferring wetter environments. It produces an annual crop of stems that emerge early in the season from overwintering rhizomes. In a mature stand, these stems close canopy rapidly and shade out any weeds, thus minimizing the need for herbicides. The canes are allowed to senesce and dry in the field providing (i) reduced fertilizer demand as mobile nutrients are recycled back to the rhizome for next year's growth, (ii) a cleaner fuel as minerals are translocated or leached from the standing biomass, and (iii) dry feedstock that can be harvested on demand as weather allows. Only recently have *M.* × *giganteus* field trials been conducted in the United States; early results in the Midwest indicate it produces two- to fourfold more biomass than upland switchgrass, 4 to 12 ton per acre (9–27 Mg/ha), respectively, on average (Heaton et al., 2008).

The high productivity, nutrient use efficiency, and lodging resistance (ability to remain upright as the plant matures) of *M.* × *giganteus* coupled with its disease and cold tolerance have stimulated considerable interest and research in *Miscanthus.* Of varieties available on the market today, *M.* × *giganteus* is the best candidate for bioenergy in temperate zones, but efficient crop practices are still in development and plant stock is in short supply. Because this hybrid cannot produce seed, it must be planted from rhizome pieces, an expensive and slow process that has been limited by low initial quantities of source material in the United States. Rhizomes are available for purchase and/or custom planting in the United Kingdom, where growers cooperate and contract to sell feedstock directly to electricity generators.

Commercial and public breeding programs are now underway for *Miscanthus* in the United States and the EU, and new varieties are expected on the market in the next five years. Genomic research and breeding of near relatives such as sugarcane and sorghum will facilitate variety development, although it is not clear if new varieties of *Miscanthus* will be planted from vegetative material or from seed. In the United States, agronomic practices and environmental impacts of *M.* × *giganteus* are being studied most prominently in the Midwest at the University of Illinois. Interest in *Miscanthus* and other previously noncrop species has created a need for risk assessment of potential bioenergy crops as invasive species. Forage introductions offer successful and cautionary examples of plant introduction that should serve as a reference when evaluating bioenergy crops.

Napiergrass [*Pennisetum purpureum* (L.) Schum.] is capable of producing more biomass than most other perennial grasses (3–5 ton/acre [7–11 Mg/ha] without nitrogen fertilizer and 7–14 ton/acre [16–31 Mg/ha] with moderate nitrogen fertilization), making it a good candidate for biofuel crop development in tropical regions. Napiergrass is not tolerant of frost and grows best under daytime highs of 85 to 95°F (30–35°C). Napiergrass varieties are single genotypes that are increased and propagated by stem cuttings or rhizomes. Napiergrass can be crossed with annual

Midwinter harvest of *Miscanthus* canes helps to ensure soluble carbohydrates and nitrogen reserves have been completely mobilized into roots and rhizomes for spring recovery and minimizes the ash and mineral content of above-ground biomass. (Photo by E. Heaton)

members of the *Pennisetum* genus to create stable seed-propagated hybrids with high biomass yield and quality.

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is a perennial grass suitable for hay or pasture production in warm, humid regions. Bermudagrass is very long-lived and has produced more dry matter biomass than switchgrass or napiergrass over a six-year period in Georgia. As a potential bioenergy crop, bermudagrass has a relatively low growth profile, necessitating multiple harvests, and relatively high fossil fuel inputs, and it requires relatively high amounts of nitrogen fertilizer per unit of biomass production. Nevertheless, superior varieties of bermudagrass, with high biomass yield and efficient conversion of cellulosic biomass to liquid fuels, may have utility in certain climates and production systems in which bermudagrass is particularly well adapted.

Cool-Season Grasses

While there is considerable effort devoted to development of warm-season grasses as bioenergy feedstocks, a small number of cool-season grasses have demonstrated potential for high biomass yields and relatively broad adaptation under relatively low-input production systems, including infrequent harvest management that would reduce fossil-fuel inputs. In humid regions, reed canarygrass (*Phalaris arundinacea* L.) is widely adapted to habitats prone to flooding and/or frequent waterlogging. Reed canarygrass is capable of producing high biomass yields with application of municipal wastewater, eliminating the need for fossil-fuel-based nitrogen fertilizers. In dryland regions where drought and highly saline soils are frequently encountered, several *Leymus* species have demonstrated the broad adaptation, drought and salinity tolerance, and relatively high biomass yields that make them potential candidates for development of dedicated bioenergy feedstocks.

ALFALFA

Legumes offer a distinct advantage over grasses because they are capable of fixing nitrogen from the atmosphere and do not require additional fossil-fuel inputs in the form of nitrogen fertilizer. Alfalfa (*Medicago sativa* L.) has received significant attention as a cellulosic biofuel crop, largely because of its broad adaptation range, its perenniality, the existence of a strong seed industry and processing infrastructure, and because it offers a valuable coproduct. Alfalfa forage can be separated into stems and leaves during or after the harvest process, creating two product streams: (i) stems that can be processed for energy production and (ii) leaves that can be processed into a high-value, moderate-protein feed for livestock.

Development of alfalfa as a bioenergy crop has proceeded as an integrated approach, using a unique combination of genetics and management. The USDA-ARS in St. Paul, Minnesota, has developed unique populations of alfalfa genotypes that respond well to very low plant densities by producing large, thick stems with high biomass yield potential and relatively high lodging resistance. Tall, lodging-resistant stems offer the opportunity to reduce the number of harvests to two or three per year, significantly reducing fossil fuel inputs from the normal three or four harvests. Theoretical ethanol yields per acre were double for the biomass-type alfalfa under a low plant density and two-harvest management compared to a forage-type alfalfa under a normal plant density and a four-harvest management (326 vs. 164 gal ethanol per acre per year [499 vs. 251 L per hectare per year]).

ANNUAL CROPS

Many annual crops will be major contributors of biomass for bioenergy production. Some of the most important of these are annual grain crops used to generate two products: grain for human or livestock consumption and cellulosic biomass from stover. Corn (*Zea mays* L. ssp. *mays*), sorghum [*Sorghum bicolor* ssp. *bicolor* (L.) Moench], and pearl millet (*Pennisetum glaucum* (L.) R. Br.) are among the leading candidates in this group. Up to 50% of the dry matter in a corn crop can be harvested as stover for biomass conversion. However, caution is necessary with this practice to ensure soil quality is not compromised with stover removal. Sweet sorghums, with a high concentration of readily fermentable sugars in the stem, are the focus of much sorghum research for bioenergy production in fermentation systems. All three of these species have natural mutants, such as brown-midrib genes, that can be used by plant breeders to enhance fermentability of plant biomass.

HARVESTING, STORAGE, AND TRANSPORTATION

Biomass grasses and legumes are commodities typically harvested and marketed in raw form, with little value-added processing taking place before sale. Like any other agricultural commodity, minimizing production costs is imperative so profits can be realized. These crops are perennials, so after establishment, the main production costs will be for fertilizer, weed control, harvesting, and storage. An understanding of harvesting and storage practices for these crops is critical if costs are to be minimized.

Harvesting perennial grasses and legumes as biomass feedstocks can usually be done with the same equipment and harvesting systems used for forage crops. Most biomass crops are currently harvested by cutting, field drying, and then storing in dry bales. Although yields of biomass crops are typically two to three times those of forage crops, most forage cutting and baling equipment can be used without modification to harvest biomass crops. However, increased machine capacity may be needed as agronomic research leads to greater biomass yields.

Short field drying time promotes fewer losses and higher product quality. Field drying time is shorter for switchgrass and reed canarygrass cut in late summer or early fall than typically reported for forage crops because these biomass crops contain less moisture at cutting, and the heavy, stiff stems produce a low-density, aerated swath. Drying to baling moisture is possible in two to three days under most environmental conditions. Moisture content in standing crops of

Direct-cut, single-pass harvesting of switchgrass in late August using a forage harvester. Material was direct-ensiled in a bag silo at 36% dry matter and average particle-size of 7 mm. Loss of dry matter during 323 days in storage was 5%. (Photo by K. J. Shinners)

mature switchgrass and reed canarygrass is less than 15% when overwintered and harvested in the late spring, meaning that they could be baled at the same time as cutting, thereby reducing costs by eliminating field operations (Shinners et al., 2006). However, at this time, no machine is available that can simultaneously cut and bale grasses.

High bale density reduces the number of bales that need to be handled and maximizes the weight that can be transported. The maximum legal volume and weight of trucks on most roads in the United States limits bale density to about 15 lbs/ft^3 (732 kg/m^3). Because biomass grasses are most often harvested quite mature, they have large, stiff stems that resist the compressive forces of both large round and square balers. Bale density of switchgrass and reed canarygrass is about 10 to 20% less than typically found with alfalfa or forage grass crops.

Biomass grasses can alternatively be direct cut and stored by ensiling because the standing moisture is often ideal for ensiling without wilting. Harvesting this way eliminates field drying, raking, baling, and bale handling, greatly reducing harvesting costs. The crop can be finely chopped at harvest as a form of mechanical pretreatment, which adds to the feedstock value. Storage losses of direct-cut and ensiled switchgrass and reed canarygrass were less than 6% of dry matter (Shinners et al., 2006). Reduced costs from eliminating field operations must more than offset the higher costs of storing and transporting the high-moisture and low-bulk density feedstock. Transport costs for hauling dry hay vary widely depending on the type of bale, distance hauled, and current fuel prices. In 2008, costs for hauling hay ranged from approximately \$2 to \$6 per loaded mile with an average of approximately \$3.50, not including the costs of loading and unloading. Hauling silage large distances beyond the field is rare because of the high moisture content of silage.

Producing liquid fuels from biomass in a cost- and time-efficient manner requires pretreatment. High-moisture, direct-cut biomass opens up the option of pretreatment at the farm. In-storage pretreatments are limited to ambient temperature and pressure conditions, resulting in reaction times that can be on the order of months. Significantly increasing the degradability of cellulose and hemicellulose in biomass through in-storage pretreatments should add value—by either allowing milder pretreatment or possibly eliminating the need for pretreatment at the biorefinery.

Forage crops are typically cut multiple times per season to ensure high nutritional quality for livestock. However, these quality metrics are not critical for biomass crops, meaning cuttings can occur once or twice per season to decrease fossil-fuel inputs. Multiple cuttings of biomass grasses typically result in 30 to 40% greater annual yield than single-cut systems (Shinners et al., 2006). However, multiple cuttings require greater nitrogen application to compensate for the nitrogen removed in the midseason harvest(s) and may adversely affect productivity and persistence (Parrish and Fike, 2005). Biomass crops have lower value than forage crops, so any added yield from multiple cuttings must substantially offset the added costs of extra harvests and increased nitrogen application. Harvest costs can be considerable, ranging from $20 to $50 per ton ($22–55 per metric ton) of dry hay.

Harvest timing affects yield, stand persistence, weed control, and feedstock chemical composition. With a single-cut system, there is a reduction in yield of 1 to 15% when biomass grass harvest is delayed from late summer to late fall (Shinners et al., 2006). When the biomass grasses are left standing over winter and harvested in the spring, dry matter yields are reduced by 20 to 40% due to senescence (death due to maturation) of leaf tissue and lodging. However, spring harvest reduces concentration of ash and undesirable minerals, which is advantageous for combustion.

Changes in crop content and/or quality during storage are typically due to dry-matter losses in the nonstructural components; these components usually have little impact on the energy yield of the feedstock. However, the producer will realize an income loss by any loss in feedstock dry matter during storage. Dry matter losses in round bales of biomass grass hay stored outdoors were 2 to 15% of dry matter depending on precipitation level, type of bale wrap, and ground preparation (Shinners et al., 2006). Bales stored indoors or under a bale tarp typically undergo dry matter losses of less than 3%.

Stems are high in the structural components desired in a biomass feedstock, whereas leaves have high nonstructural and protein composition, which is desirable as digestible animal feed. Harvesting the high-fiber lower portion of the plant separately from the high-digestible upper portion is known as *harvest fractionation*. Harvest fractionation can be accomplished by stripping the leaves and upper stems from the standing plant using a tined stripping rotor (Shinners et al., 2007). The stripped fraction can be chopped and directly ensiled. The stem fraction is simultaneously cut and then field dried and either chopped or baled. This process works with either legumes or biomass grasses. The protein difference between the fractions is much greater for legumes than for grasses. The at-harvest moisture content of grasses allows direct ensiling of both fractions, but the leaves and upper portions of the legume plant are usually too high in moisture to directly ensile safely without amendments or additives.

INCENTIVES, ENERGY BALANCE, AND ECONOMICS

Long-term viability of grassland-based bioenergy production systems will be realized only if they are profitable, possess high net energy yields (energy production per acre), and maintain or improve environmental quality. Likewise, initial adoption of grassland-based systems may depend on financial incentives—both public and private—to encourage a transition to cellulosic bioenergy production.

ECONOMIC LIMITATIONS AND INCENTIVES

Among the portfolio of grassland-based bioenergy sources, switchgrass has much potential as an economically feasible feedstock for the production of cellulosic ethanol. Field-scale research using known farm inputs and actual harvested switchgrass yields determined that switchgrass could be delivered to the farm gate for $60 per ton ($66 per metric ton) (Perrin et al., 2008). New cultivars, improved production practices, and expanded switchgrass markets may reduce farm-gate costs by as much as 20%, making switchgrass production even more economically competitive relative to other agricultural production practices.

Energy Balance and Conversion of Marginal Land

In addition to economic viability, the energy efficiency of grassland-based bioenergy production systems is a key factor in determining their long-term sustainability. Energy produced from renewable carbon sources is held to a different standard than energy produced from fossil fuels, in that renewable fuels must have highly positive energy values. Recent field-scale research has found switchgrass production to excel in this regard. Switchgrass fields on 10 farms in Nebraska, South Dakota, and North Dakota produced 6.4-fold more renewable fuel than nonrenewable fuel consumed over a five-year period (Schmer et al., 2008). Furthermore, estimated on-farm net energy yield of moderate-input switchgrass was nearly twice that for human-made prairies and six times greater than for low-input switchgrass. Such results underscore the energetic and environmental benefits of managing switchgrass for bioenergy production in the Central and Northern Great Plains.

Should grassland-based bioenergy production systems be implemented in the United States, conversion of marginal land from annual row crops to switchgrass production could exceed 10% in some regions. Consequently, it is important to understand the feasibility and production potential of marginal sites. Research in Nebraska found potential ethanol yield of switchgrass to average 371 gallons per acre (3470 L per hectare), which was equal to or greater than the potential ethanol yield of no-till corn on a dryland site with marginal soils (Varvel et al., 2008). Although similar comparisons are needed in other locations throughout the United States, the parity in potential ethanol yield between switchgrass and annual row crops on marginal sites is encouraging, particularly since the former will likely enhance ecosystem services more rapidly and significantly than on productive sites.

Carbon Sequestration and Carbon Credits

Agricultural land is increasingly looked on to provide key ecosystem services for a growing human population. In the context of bioenergy production, grasslands not only supply energy, but they concurrently conserve soil and water, increase wildlife habitat, and sequester carbon. Carbon sequestration, in particular, is an ecosystem service that can provide a financial benefit to producers while improving soil quality and mitigating agriculture's contribution to global climate change.

Grassland-based bioenergy production systems possess significant potential to take up carbon dioxide from the atmosphere and sequester it in soil. As an example, carbon sequestration by switchgrass has been found to be nearly ubiquitous across a broad range of growing conditions, and at rates higher than most agricultural production systems. Switchgrass also sequesters carbon deep in the soil profile (below 1 foot, or 30 cm) owing to a prolific and extensive root system that can extend to a depth of 9 feet (2.7 m). Such "deep" sequestration of carbon by switchgrass makes its storage more permanent, as return of carbon back to the atmosphere as carbon dioxide is less from deeper depths than from depths near the soil surface. Similar to deep-rooted perennial grasses, perennial legumes (such as alfalfa) can concurrently be used for bioenergy production while sequestering carbon in soil. An added benefit with perennial legumes is their capacity to "fix" nitrogen in soil, thereby reducing fertilizer requirements.

The value of sequestered carbon by grassland-based bioenergy production systems extends beyond on-site improvements in soil quality and reduced input requirements. Agricultural producers in the United States have the opportunity to earn additional income through involvement in carbon trading programs for acres they plant to perennial grass or alfalfa. Such programs provide a framework for multinational corporations, utility and power

The perennial native switchgrass provides continuous cover on highly erodable land. (Photo by M. Schmer)

generation companies, and other private and public organizations to offset their carbon dioxide emissions by purchasing carbon credits from entities known to achieve net carbon storage. Although such programs are currently voluntary, they have achieved considerable success in North America. Since 2003, over 6 million acres (2.4 million ha) of approved conservation practices have been enrolled in carbon credit programs throughout North America (Liebig et al., 2008).

Natural Resource Conservation Programs

Publicly funded conservation programs have a long history of success in restoring previously degraded lands throughout the United States. Establishing close-seeded or solid stands of perennial grasses and legumes as restoration treatments has been a cornerstone of many conservation programs, as these species act to recover ecosystem attributes degraded through long-term cultivation. In this context, planting appropriate perennial species for bioenergy production can meet conservation goals while concurrently providing energy. Furthermore, producers can receive publicly funded assistance for doing so.

The Environmental Quality Incentives Program (EQIP) is a voluntary conservation program that promotes agricultural production and environmental quality as compatible goals. Facilitated by the USDA, producers enrolled in EQIP contracts can receive incentive payments and cost-shares (up to 75%) to implement conservation practices, such as planting perennial grasses and legumes. With incentive payments provided up to 3 years and contracts lasting a maximum of 10 years, this program can contribute to establishing and maintaining multifunctional grassland-based bioenergy production systems throughout the United States.

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